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Positional Information: Knowing Where You Are in a Limb

Dispatch

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In the regenerating amphibian limb, positional information has traditionally been considered in terms of short-range cell–cell interactions, not long-range diffusion gradients. A molecule discovered in a differential screen of regenerating limbs turns out to be precisely such a cell surface component, the newt ortholog of mouse CD59.

There can be few clearer examples of exactly what positional information is all about than amphibian limb regeneration. When the hand of an amphibian is cut off then the hand regenerates beginning at the level of the carpals, but when the whole arm is cut off then a new arm regenerates beginning from the level of the humerus (Figure 1a). The same is true at any intermediate level. Therefore the cells which begin the regeneration process must know exactly where they are in the limb, because they know which parts are missing.

The cells which measure this positional informa -tion are known as blastemal cells, and they arise at the amputation plane by dedifferentiation of the internal tissues that have been damaged: cartilage or bone, muscle, dermis and connective tissue. The mound of blastemal cells so generated is covered by the epi-dermis, which rapidly migrates over the cut stump to heal the wound. As a result of dedifferentiation, blastemal cells assume an embryonic, multipotent phenotype and begin rapid division to replace the lost tissues.

It is these blastemal cells which must have some measure of positional information so that they know what to replace, but what form does this positional information take? Most developmental biologists would assume that positional information is determined by a gradient of an extracellular 'morphogen', such as retinoic acid, Decapentaplegic, TGFβ, Squint, Wingless or BMP [1–3]. In these cases, a group of cells — the 'source' — produces the morphogen, which diffuses across a field of cells forming a concentration gradient to which the cells in the field differentially respond. But the idea of a diffusion gradient of an extracellular molecule has never been popular in regeneration studies for three reasons.

Firstly, there is a size issue. Developing fields with diffusing morphogens tend to be a few hundred micrometres in size. For example, the progress zone of the chick limb bud is 300μm across [4] and TGFβ diffuses 100–200μm across a field of cells in the *Xenopus* animal cap [2]. Indeed, theoretical considerations suggested that the maximum distance over which a morphogen gradient could operate is 1mm [5]. The blastema of an adult axolotl, however, can be 5mm in each dimension, giving a volume 4000 times bigger than a limb bud, surely too large for extracellular diffusion gradients. Secondly, the influential clockface model [6], which united theoretical concepts from insects and amphibians, proposed that cell interactions take place very locally between immediately adjacent cells, rather than over long distances of many cell diameters as in the case of a morphogen gradient. Thirdly, the assays of positional information which have been devised in limb regeneration studies revealed that cells have information on their surfaces which is of a positional nature.

This positional behavior can be easily demonstrated in the circumferential axes of the limb: the anteroposterior axis from thumb to little finger, and the dorsoventral axis from the palmar surface to top of the hand. When the blastema is cut off the stump, rotated 180° and stuck back on, then in about half of the cases supernumerary limbs are regenerated from the join between the blastema and the stump [7]. But in the other half of the cases, the blastema slowly derotates over a period of several days and assumes its original location (Figure 2a). The whole blastema undergoes this remarkable movement which must surely occur as a result of cell–cell interactions.

Similarly, along the proximodistal axis, when a distal blastema, regenerating a hand, is grafted adjacent to a proximal blastema, regenerating a whole limb, so that the blastema cells are in contact, then the distal blastema does not integrate into its new location and generate, for example a hand protruding from the shoulder. Instead, it is displaced during the regrowth of the proximal blastema until it reaches its precise level of origin on the proximodistal axis — the hand — where it integrates and gives rise to a lateral regenerate (Figure 2b) [8]. Blastemas *in vitro* also display cell surface differences, which is surely related to these *in vivo* properties. In culture, proximal blastemas always engulf distal blastemas, and on the basis of these properties it was suggested that there is a gradient of cell adhesivity along the proximodistal axis [9].

An important tool for analyzing the nature of positional information came with the discovery that retinoic acid can change positional information in a precise concentration-dependent fashion [10]. As described above, normally the blastema regenerates precisely what was removed by amputation, but if a distal blastema is treated with retinoic acid then it regenerates, not a hand, but a whole limb instead (Figure 1b). Intermediate results are obtained with intermediate concentrations of retinoic acid, and it was clear that retinoic acid gradually changes the positional information of blastemas over the surprisingly small concentration range of about 2.5-fold. If positional information is located at the cell surface then these cell surface properties should also change

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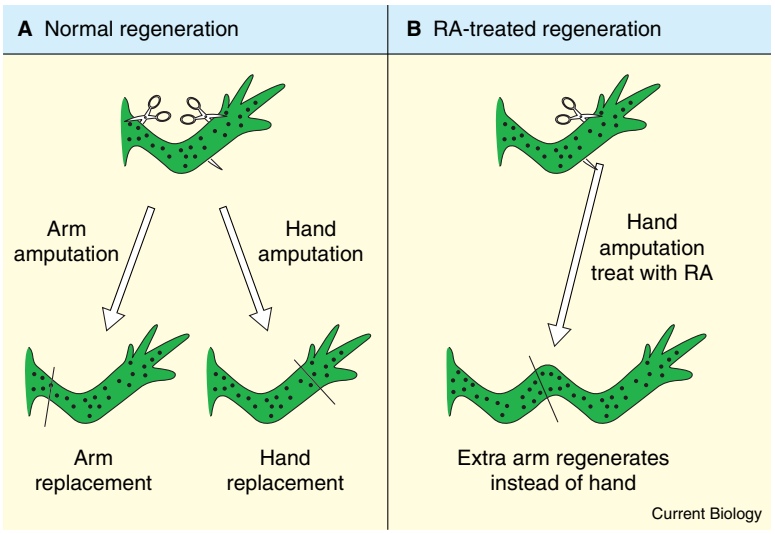

Figure 1. Positional information in amphibian limb regeneration.

The drawings show (A) the results of normal regeneration from two different levels, and (B) the effects of retinoic acid on regeneration.

with retinoic acid treatment, and indeed that was also the case. A retinoic acid-treated blastema grafted to a proximal site no longer relocated to distal levels, but stayed at proximal levels [8].

With the advent of subtractive screening using cDNA libraries, the stage was set for a screen comparing gene expression in untreated and retinoic acid-treated distal blastemas, and this is precisely what has been successfully performed in the new work of da Silva *et al.* [11]. The hope was that this screen would reveal molecules that are altered co-ordinately with the retinoic acid-induced positional change, although in view of the small concentration range over which retinoic acid has its effects, only small expression differences might be expected. The subtractive screen was optimised to take this into account.

From four repeat subtractions, 151 upregulated and 100 downregulated candidate genes were identified [11]. To be a candidate for further analysis, a gene had to show a difference in expression between proximal and distal blastemas, be upregulated or downregulated by retinoic acid, and encode a cell-surface protein. Amazingly, only one clone satisfied all these criteria, and this was termed *Prod 1*. *Prod 1* encodes a glycosyl-phosphatidylinositol (GPI)-anchored cell surface protein, which da Silva *et al.* [11] suggest is the newt orthologue of the mammalian cell surface protein CD59. Although the overall sequence identity is not high, the conserved motifs and secondary structure suggest that Prod 1 really is a CD59 orthologue.

Prod 1 is expressed by blastemal cells at a 1.7-fold higher level in proximal than distal blastemas, and it

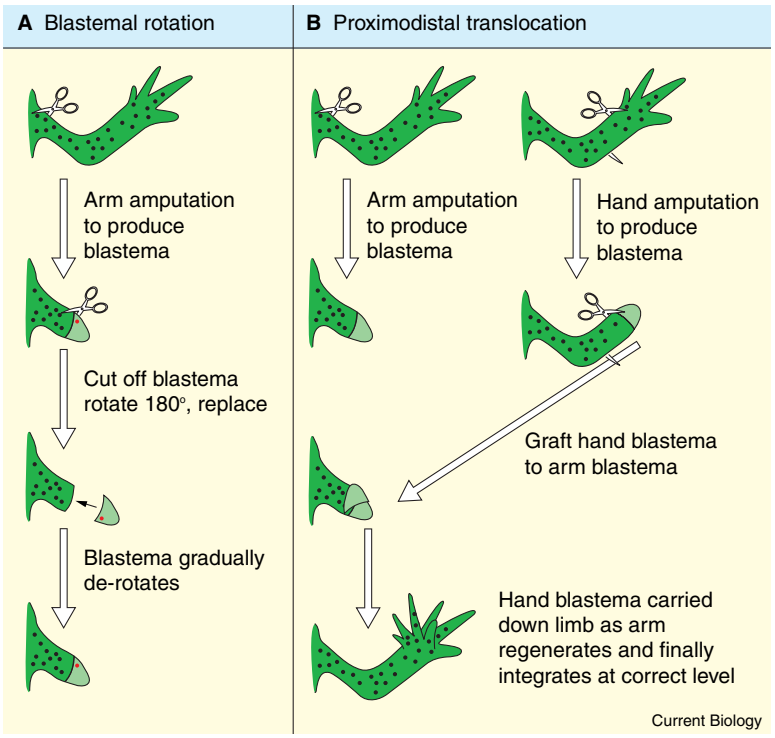

Figure 2. Cell-surface interactions and positional information.

The drawing illustrates experiments that have shown that blastemal cells can recognise disparities in positional information by cell-surface interactions.

is upregulated 15-fold by retinoic acid treatment. Surprisingly this same level of difference between proximal and distal is also present in the normal, unamputated limb, suggesting that the assessment of positional information is not a property only of dedifferentiated blastemal cells. When proximal and distal blastemas were confronted in culture and the proximal blastema engulfed the latter, then an antibody against the Prod 1 protein showed strong immunoreactivity to the proximal blastema and low immunoreactivity to the distal blastema. When phospholipase C, which removes GPI-linked surface molecules, was placed into the culture medium, then no engulfment took place. Most importantly, engulfment also failed to take place when either of two different Prod 1 antibodies was added to the medium.

So how does Prod 1 work? The behavior of blastemal cells described above shows that they are locally activated after confrontation by cells that differ in cell-surface levels of Prod 1 (at least). Da Silva *et al.* [11] suggest that Prod 1 might act between cells as a homodimer which prevents activation of cytoplasmic signaling cascades. Proximal cells with more Prod 1 on their surfaces would therefore have some spare un-homodimerized receptors, which would be available for activation leading to the cellular responses of engulfment (*in vitro*) or proliferation (*in vivo*). However it acts, this GPI-linked cell surface molecule is a remarkable molecular realization of theoretical concepts based on cell–cell interactions.

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